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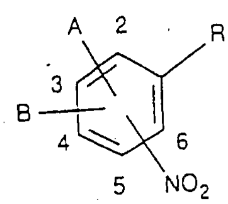

(I)

(57) Abstract

The invention provides nitroaniline derivatives represented by general formula (I) where the nitro group is substituted at any one of the available benzene positions 2-6; where R and A separately represent the groups NO_2, CN, $COOR^1$, $CONR^1R^2$, $CSNR^1R^2$ or $SO_2NR^1R^2$ and A is substituted at any one of the available benzene positions 2-6; where B represents $N(CH_2CH_2halogen)_2$ or $N(CH_2CH_2OSO_2R^3)_2$ substituted at any one of the available benzene positions; and where R^1, R^2 and R^3 separately represent H, or lower alkyl optionally substituted with hydroxyl, ether, carboxy or amino functions, including cyclic structures, or R^1 and R^2 together with the nitrogen form a heterocyclic structure, and pharmaceutical preparations containing them. These compounds have activity as hypoxia-selective cytotoxins, reductively-activated prodrugs for cytotoxins, hypoxic cell radiosensitisers, and anticancer agents.

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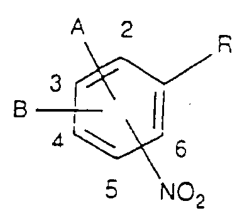
NITROANILINE DERIVATIVES AND THEIR USE AS ANTI-TUMOUR AGENTS

Alkylating agents are an important class of anticancer drugs, which express their cytotoxic and antitumour effects by forming adducts with cellular DNA (Garcia et al., *Biochem. Pharmacol.*, 1988, **37**, 3189).

The present invention relates to novel nitroaniline-based alkylating agents having activity as hypoxia-selective cytotoxins, reductively-activated prodrugs for cytotoxins, hypoxic cell radiosensitisers, and anticancer agents, to methods of preparing the novel compounds, and to the use of these compounds as antitumour agents.

In one aspect, the present invention relates to the class of nitroaniline derivatives represented by the general formula (I);



(I)

where the nitro group is substituted at any one of the available benzene positions 2-6; where R and A separately represent the groups NO_2, CN, $COOR^1$, $CONR^1R^2$, $CSNR^1R^2$ or $SO_2NR^1R^2$ and A is substituted at one of the available benzene positions 2-6; where B represents $N(CH_2CH_2halogen)_2$ or $N(CH_2CH_2OSO_2R^3)_2$ substituted at any one of the available benzene positions; and where R^1, R^2 and R^3

separately represent H, or lower alkyl optionally substituted with hydroxyl, ether, carboxyl or amino functions, including cyclic structures, or R^1 and R^2 together with the nitrogen form a heterocyclic structure.

Where R^1, R^2 and/or R^3 represent lower alkyl, the group may contain from 1 to 6 carbon atoms.

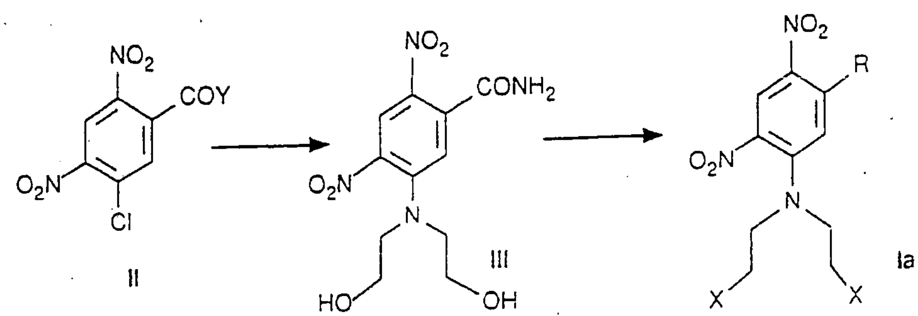
Where R^1, R^2 and/or R^3 represent groups containing tertiary amines, the N-oxides of those tertiary amine moieties are also included.

The compounds of formula (I) have cytotoxic and antitumour activity, and are useful as hypoxia-selective cytotoxins, reductively-activated prodrugs for cytotoxins, hypoxic cell radiosensitisers, and anticancer agents.

Some of the compounds of formula (I) form pharmaceutically-acceptable addition salts with both organic and inorganic acids, and these addition salts also form part of the present invention. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic and the like. Some of the compounds of formula (I) form pharmaceutically-acceptable addition salts with both organic and inorganic bases, and these addition salts also form part of the present invention. Examples of suitable bases for salt formation are sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine and the like.

The compounds of formula (I) and the acid or base addition salts and the N-oxides thereof may be prepared by the processes outlined in the following Schemes 1 to 7.

Specific but non-limitative examples of the processes outlined in Schemes 1 to 7 are given hereinafter in Examples A to H respectively

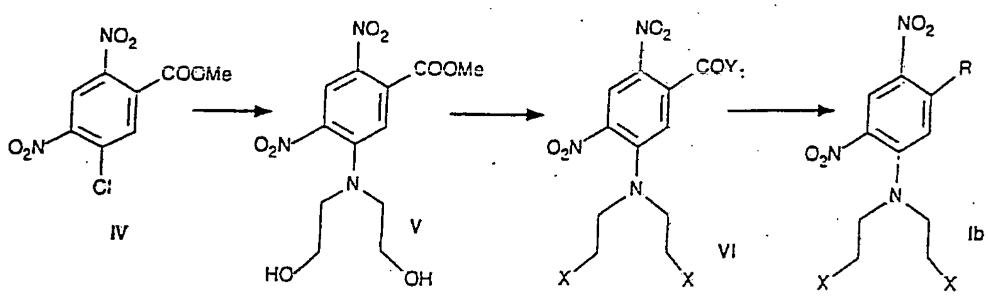


Scheme 1

Y = OH, NH_2

X = OSO_2Me, halogen.

R = $CONH_2$

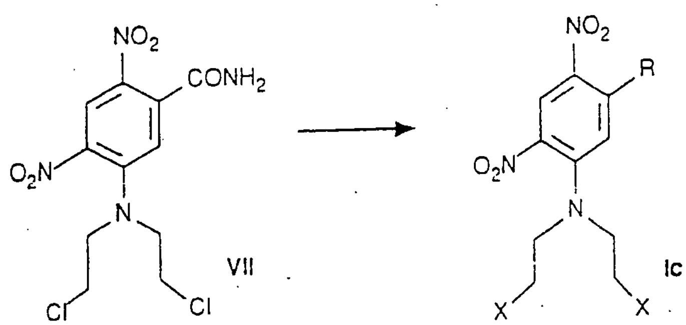


Scheme 2

$X = OSO_2Me$, halogen

$Y_1 = Cl$

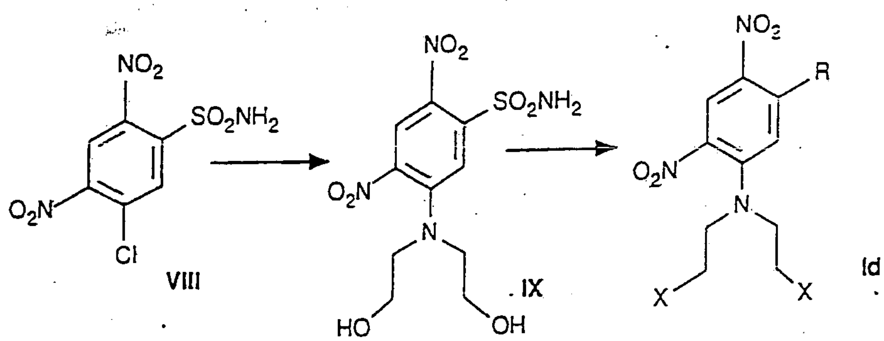
$R = COOR^1, CONR^1R^2$



Scheme 3

X = Cl

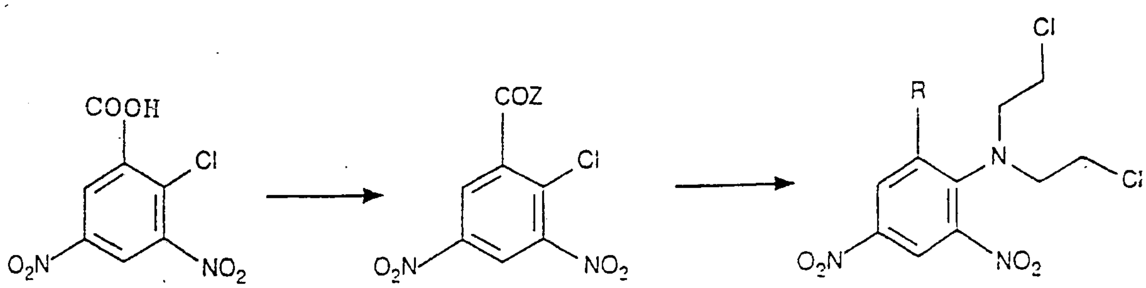
R = CN or $CSNH_2$



Scheme 4

$X = OSO_2Me$, halogen

$R = SO_2NH_2$

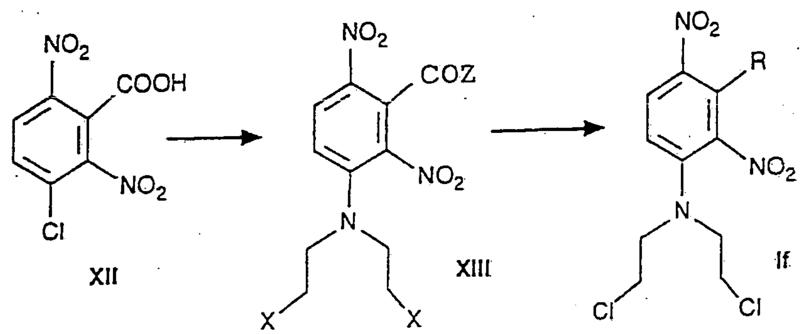


X XI Ie

Scheme 5

Z = Cl, NH_2, NR^1R^2

R = $CONH_2$, $CONR^1R^2$

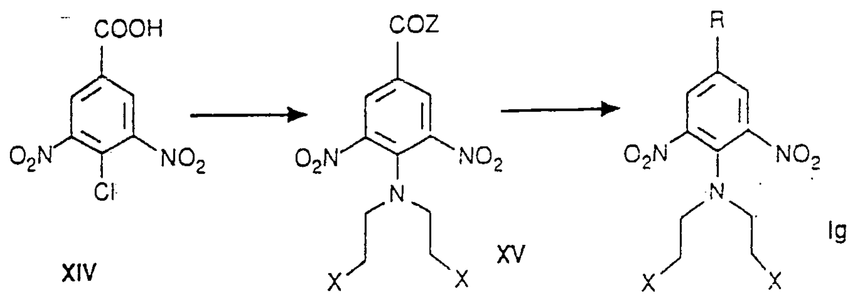


Scheme 6

X = OH, Cl

Z = OEt, OH, Cl

R = $CONR^1R^2$



Scheme 7

$X = OH, Cl$

$Z = OMe, OH, Cl$

$R = CONR^1R^2$

The invention also relates to the preparation of compounds of the general formula I, the acid or base addition salts and the N-oxides thereof, by a process as outlined in any one of Schemes 1-7 above or by an obvious chemical equivalent thereof. The invention includes also those forms of the preparation processes according to which a compound obtainable as intermediate at any stage of the process is used as starting material and the remaining steps are carried out, or a starting material is used in the form of a derivative or salt or, especially, is formed under the reaction conditions.

The following Table 1 sets out physicochemical data for 24 compounds within the general formula (I), representative of it, and preparable by the processes of the invention.

Table 1. Analytical and physicochemical data for nitroaniline mustards of general formula 1.

No.	A	NO_2	B	R	mp (°C)	formula	analyses[a]
1	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONH_2$	109-111	$C_{11}H_{12}Cl_2N_4O_5$	C,H,N,Cl
2	2-NO_2	4	5-$N(CH_2CH_2Br)_2$	$CONH_2$	126-128	$C_{11}H_{12}Br_2N_4O_5$	C,H,N
3	2-NO_2	4	5-$N(CH_2CH_2I)_2$	$CONH_2$	170-172	$C_{11}H_{12}I_2N_4O_5$	C,H,N,I
4	2-NO_2	4	5-$N(CH_2CH_2OMs)_2$[b]	$CONH_2$	142-144	$C_{13}H_{18}N_4O_{11}S_2$	C,H,N,S
5	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	COOMe	136.5-138	$C_{12}H_{13}Cl_2N_3O_6$	C,H,N,Cl
6	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	COOH	125-127	$C_{11}H_{11}Cl_2N_3O_6$.½EtOAc	C,H,N
7	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	CN	127	$C_{11}H_{10}Cl_2N_4O_4$	C,H,N
8	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	CONHMe	180.5	$C_{12}H_{14}Cl_2N_4O_4$	C,H,N,Cl
9	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONMe_2$	130.5	$C_{13}H_{16}Cl_2N_4O_5$	C,H,N
10	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	CONX[c]	140-142	$C_{15}H_{18}Cl_2N_4O_6$	C,H,N
11	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONH(CH_2)_2NMe_2$.HCl	85-90	$C_{15}H_{21}Cl_2N_5O_5$.HCl	C,H,N
12	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONH(CH_2)_2NX$[c].HCl	119-120	$C_{17}H_{23}Cl_2N_5O_6$.HCl	C,H,N,Cl
13	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CSNH_2$	166-167	$C_{11}H_{12}Cl_2N_4O_4S$	C,H,N,Cl
14	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONH(CH_2)_2N(O)Me_2$	165-169	$C_{15}H_{21}Cl_2N_5O_6$.HCl	C,H,N,Cl
15	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONH(CH_2)_2COOH$	150-153	$C_{14}H_{16}Cl_2N_4O_7$	C,H,N
16	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONHCH_2CH_2OH$	157-158	$C_{13}H_{16}Cl_2N_4O_6$	C,H,N,Cl
17	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	$CONHCH_2CH(OH)CH_2OH$	gum	$C_{14}H_{18}Cl_2N_4O_7$	C,H,N,Cl
18	2-NO_2	4	5-$N(CH_2CH_2Cl)_2$	SO_2NH_2	153-154	$C_{10}H_{12}Cl_2N_4O_6S$	C,H,N,S
19	3-NO_2	5	2-$N(CH_2CH_2Cl)_2$	$CONH_2$	123-124	$C_{11}H_{12}Cl_2N_4O_5$	C,H,N,Cl
20	3-NO_2	5	2-$N(CH_2CH_2Cl)_2$	$CONH(CH_2)_2NMe_2$	115-118	$C_{15}H_{21}Cl_2N_5O_5$	C,H,N,Cl
21	2-NO_2	6	5-$N(CH_2CH_2Cl)_2$	$CONH_2$	141-141.5	$C_{11}H_{12}Cl_2N_4O_5$	C,H,N,Cl
22	2-NO_2	6	5-$N(CH_2CH_2Cl)_2$	$CONH(CH_2)_2NMe_2$.HCl	180-182	$C_{15}H_{21}Cl_2N_5O_5$.HCl	C,H,N,Cl
23	3-NO_2	5	4 $N(CH_2CH_2Cl)_2$	$CONH_2$	113-116	$C_{11}H_{12}Cl_2N_4O_5$	C,H,N,Cl
24	3 NO_2	5	4-$N(CH_2CH_2Cl)_2$	$CONH(CH_2)_2NMe_2$.HCl	145-148	$C_{15}H_{21}Cl_2N_5O_5$.HCl.H_2O	C,H,N

Footnotes for Table 1 [a] Analyses for all listed elements within ± 0.4%. [b] OMs = OSO_2CH_3. [c] X = morpholide : $(CH_2CH_2)_2O$.

The following Examples A to H illustrate the preparation of compounds representative of the general formula (I), and specifically the compounds listed in Table 1.

Example A : Preparation of 5-[N,N-bis(2-methanesulfonoxyethyl)amino]-2,4--dinitrobenzamide (4) (Ia: X = OSO_2Me; R = $CONH_2$) and analogues by the method outlined in Scheme 1.

Treatment of 5-chloro-2,4-dinitrobenzoic acid (II: Y = OH) [Goldstein, H.; Stamm, R. *Helv. Chim. Acta.*, **1952**, *35*, 1330-1332] with $SOCl_2$ followed by ammonia gave 5-chloro-2,4-dinitrobenzamide (II: Y = NH_2), mp 210 °C (dec) [Goldstein, H.; Stamm, R. *Helv. Chim. Acta.*, **1952**, *35*, 1330-1332 report mp 212 °C] . ^1H NMR (CD_3COCD_3) δ 8.68 (s, 1 H, H-3), 8.02 (s, 1 H, H-6), 7.50 (br, 2 H, $CONH_2$). This amide was heated with diethanolamine in dioxan to give 5-[N,N-bis(2--hydroxyethyl)]amino-2,4-dinitrobenzamide (III) (45% yield), mp (EtOAc) 176-178°C. ^1H NMR (CD_3SOCD_3) δ 8.47 (s, 1 H, ArH3), 8.07, 7.73 (2 br, 2 H, $CONH_2$), 7.35 (s, 1 H, ArH6), 4.80 (m, 2 H, OH), 3.83-3.20 (m, 8 H, CH_2N, CH_2O). Anal. ($C_{11}H_{14}N_4O_7$) C,H,N.

Methanesulfonyl chloride (5.17 ml, 0.067 mol) was added dropwise to a solution of the above amide diol (10.00 g, 0.032 mol) in dry pyridine (150 mL) at 0 °C. After a further 10 minutes at 0 °C, the solution was stirred at 20 °C for 30 minutes, and then volatiles were removed under reduced pressure at below 40 °C. The residue was partitioned between EtOAc and water, and the organic residue was chromatographed on silica gel. Elution with EtOAc gave foreruns, followed by 5--[N,N-bis(2-methanesulfonoxyethyl)amino]-2,4-dinitrobenzamide (4) (Ia: X = OSO_2Me; R = $CONH_2$) (11.05 g, 74%), mp (sealed tube : ex EtOAc/petroleum ether) 60 °C. ^1H NMR(CD_3COCD_3) δ 8.54 (s, 1 H, H-3), 7.65 (s, 1 H, H-6), 4.49 (t, J = 5.3 Hz, 4 H, $CH_2OSO_2CH_3$), 3.89 (t, J = 5.3 Hz, 4 H, CH_2N), 3.09 (s, 6 H, SO_2CH_3), 2.90 (br s, NH_2). ^{13}C NMR δ 167.01 ($CONH_2$), 148.56 (C-5), 140.45, 138.44 (C-2,4), 138.57 (C-1), 124.92, 123.06 (C-3,6), 67.65 ($CH_2OSO_2CH_3$), 52.02 (CH_2N), 37.23 (SO_2CH_3). Anal. ($C_{13}H_{18}N_4O_{11}S_2$) C,H,N,S.

The above dimesylate (4) (15.0 g, 31.9 mol) was dissolved in dry DMF (150 mL). The solution was treated with solid LiCl (20 g), warmed to 100° C with vigorous stirring for 1h, and then concentrated to dryness. The residue was partitioned between EtOAc and water, and the organic portion was washed well with 3N HCl, worked up to give a waxy solid, and chromatographed on silica. Elution with EtOAc/petroleum ether (1:1) gave foreruns, while EtOAc/petroleum ether (3:2) gave 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (1) (Ia, X = Cl, R = $CONH_2$) (7.51 g, 67%)which crystallised from EtOAc/petroleum ether as small orange needles, m.p. 109-111° C, ^{1}H NMR (CD_3COCD_3)δ 8.52 (s, 1H, H-3), 7.58 (s, 1H, H-6), 7.10 (br, 2H, $CONH_2$), 3.89-3.81 (m, 8H, NCH_2CH_2Cl). ^{13}C NMR δ 167.10 ($CONH_2$), 148.31 (C-5), 140.08 (C-2), 138.84 (C-1), 138.20 (C-4), 124.94, 122.24 (C-3,6), 54.25 (CH_2N), 42.04 (CH_2Cl) $^M/z$ 354,352,350 (M+, 2%), 303,301 (67,24%),222(25),185(26),171(29),100(74),65(25),63(56),56(57),36(100). Found M+ 354.0124; 352.0123; 350.0189. Anal. ($C_{11}H_{12}Cl_2N_4O_5$) C, H, N, I.

A solution of the above dimesylate (4) (1.00 g, 2.12 mmol) in dry DMF (50 mL) was treated with solid NaBr (10.0 g), warmed to 120 °C with vigorous stirring for 15 min, and then concentrated to dryness under reduced pressure. The residue was partitioned between EtOAc and water, and the organic portion was washed well with 3N HCl and worked up. Chromatography of the residue on silica and elution with EtOAc/petroleum ether (1:1) gave 5-[N,N-bis(2-bromoethyl)amino]-2,4--dinitrobenzamide (2) (Ia: X = Br, R = $CONH_2$) (0.73 g, 78% yield), mp (EtOAc/petroleum ether) 126-128 °C. ^{1}H NMR (CD_3COCD_3) δ 8.53 (s, 1 H, H-3), 7.58 (s, 1 H, H-6), 3.89 (t, J = 6.6 Hz, 4 H, CH_2N), 3.73 (t, J = 6.6 Hz, 4 H, CH_2Br), 2.93 (br, 2 H, $CONH_2$). ^{13}C NMR δ 167.20 ($CONH_2$), 147.89 (C-5), 140.06, 138.26 (C-2,4), 138.75 (C-1), 124.95, 122.30 (C-3,6), 54.11 (CH_2N), 29.89 (CH_2Br). Anal. ($C_{11}H_{12}Br_2N_4O_5$) C,H,N.

A similar reaction using NaI gave 5-[N,N-bis(2-iodoethyl)amino]-2,4--dinitrobenzamide (3) (Ia: X = I, R = $CONH_2$) in 92% yield, mp (EtOAc/petroleum ether) 170-172 °C. ^{1}H NMR (CD_3COCD_3) δ 8.52 (s, 1 H. H-3), 7.55 (s, 1 H, H-6), 3.84 (t, J = 7.2 Hz. 4 H, CH_2N), 3.50 (t, J = 7.2 Hz. 4 H.

CH_2Br), 2.88 (br, 2 H, $CONH_2$). ^{13}C NMR δ 167.09 ($CONH_2$), 147.17 (C-5), 140.08, 138.33 (C-2,4), 138.74 (C-1), 124.94, 122.31 (C-3,6), 54.89 (CH_2N), 1.72 (CH_2I). Anal. ($C_{11}H_{12}I_2N_4O_5$) C,H,N,I.

Example B : Methyl 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzoate (5) (VI: X = Cl) and analogues by the method outlined in Scheme 2.

Methyl 5-chloro-2,4-dinitrobenzoate (IV) (1.24 g, 4.76 mmol) was treated with diethanolamine (1.00 g, 9.52 mmol) in dioxane (50 mL) at 50 °C for 3 h, and the residue after workup was chromatographed on silica gel. Elution with EtOAc gave methyl 5-[N,N-bis(2-hydroxyethyl)amino]-2,4-dinitrobenzoate (V) (1.52 g, 97%), mp (EtOAc/petroleum ether) 104-106 °C. ^1H NMR ($CDCl_3$) δ 8.48 (s, 1 H, H-3), 7.45 (s, 1 H, H-6), 3.95 (s, 3 H, OCH_3), 3.81 (t, J = 5.0 Hz, 4 H, CH_2OH), 3.60 (t, J = 5.0 Hz, 4 H, CH_2N), 2.20 (br, 2 H, OH). ^{13}C NMR δ 165.89 (COOMe), 147.53 (C-5), 138.67, 136.21 (C-2,4), 133.30 (C-1), 124.22, 121.65 (C-3,6), 59.34 (CH_2OH), 54.59 (CH_2N), 53.74 (OCH_3). Anal. ($C_{12}H_{15}N_3O_8$) C,H,N.

A solution of this diol (1.50 g, 4.56 mmol) and Et_3N (1.33 mL, 9.57 mmol) in dry CH_2Cl_2 (60 mL) was treated with MsCl (0.73 mL, 9.34 mmol) at 0 °C, and poured into saturated aqueous $NaHCO_3$ after 15 min. The crude dimesylate (VI: X = OSO_2Me) from workup was treated directly with excess LiCl in DMF at 120 °C for 30 min. The mixture was poured into brine, extracted with EtOAc, worked up, and chromatographed on silica gel. Elution with EtOAc/petroleum ether gave methyl 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzoate (5) (VI: X = Cl; Y^1 = OMe) (1.34 g, 80%), mp (EtOAc/petroleum ether) 136.5-138 °C. ^1H NMR ($CDCl_3$) δ 8.54 (s, 1 H, H-3), 7.34 (s, 1 H, H-6), 3.97 (s, 3 H, OCH_3), 3.68 (br s, 8 H, NCH_2CH_2Cl). ^{13}C NMR δ 165.30 ($COOCH_3$), 147.22 (C5), 139.81, 137.82 (C-2,4), 133.39 (C-1), 124.33, 121.77 (C-3,6), 53.86 (OCH_3), 53.78 (CH_2N), 40.69 (CH_2Cl). Anal. ($C_{12}H_{13}Cl_2N_3O_6$) C,H,N,Cl.

Hydrolysis of (5) with 4N KOH in p-dioxane at 20 °C for 15 min gave a quantitative yield of 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzoic acid (6) (Ib: X = Cl, R = COOH), mp (EtOAc/petroleum ether) 125-127 °C. ^{1}H NMR (CD_3COCD_3) δ 8.56 (s, 1H, H-3), 7.72 (s, 1 H, H-6), 3.89-3.86 (br, 8 H, NCH_2CH_2Cl), 3.78 (br, COOH). ^{13}C NMR δ 166.02 (COOH), 148.52 (C-5), 140.25, 138.09 (C-2,4), 134.72 (C-1), 124.87, 122.58 (C-3,6), 54.12 (CH_2N), 42.12 (CH_2Cl). Anal. ($C_{11}H_{11}Cl_2N_3O_6$.½EtOAc) C,H,N,Cl.

Treatment of (6) with excess $SOCl_2$ containing a drop of DMF under reflux for 20 min, followed by concentration to dryness and azeotroping with benzene, gave crude 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl chloride (VI: X = Cl; Y^1 = Cl). This was dissolved in Me_2CO (20 mL), cooled to 0°C, treated with excess 40% aqueous methylamine. The product was purified by chromatography on silica gel, elution with EtOAc/petroleum ether (1:1) giving N-methyl [N,N-bis(2--chloroethyl)amino]-2,4-dinitrobenzamide (8) (Ib: X = Cl, R = CONHMe), mp (EtOAc/petroleum ether) 180.5 °C. ^{1}H NMR (CD_3COCD_3) δ 8.53 (s, 1 H, ArH3), 7.75 (br, 1 H, CONH), 7.56 (s, 1 H, H-6), 3.88-3.80 (m, 8 H, NCH_2CH_2Cl), 2.90, 2.89 (2 s, 3 H, CONHMe). ^{13}C NMR δ 165.89 (CONH), 148.43 (C-5), 140.05, 138.26 (C-2,4), 138.94 (C-1), 125.05, 122.45 (C-3,6), 54.20 (CH_2N), 42.10 (CH_2Cl), 26.69 (CONHCH_3). Anal. ($C_{12}H_{14}Cl_2N_4O_5$) C.H,N,Cl.

Similar treatment of the acid chloride (VI: X = Cl; Y^1 = Cl) with ammonia or suitable amines gave :

- 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (4) (Ib: X = Cl, R = $CONH_2$) (67% yield), mp (EtOAc/petroleum ether) 109-111 °C. ^{1}H NMR (CD_3COCD_3) δ 8.52 (s, 1 H, H-3), 7.58 (s, 1 H, H-6), 7.10 (br, 2 H, $CONH_2$), 3.89-3.81 (m, 8 H, NCH_2CH_2Cl). ^{13}C NMR δ 167.10 ($CONH_2$), 148.31 (C-5), 140.08 (C-2), 138.84 (C-1), 138.20 (C-4), 124.94, 122.24 (C-3,6), 54.25 (CH_2N), 42.04 (CH_2Cl). $^M/z$ 354, 352, 350 ($M^{+\cdot}$, 2%), 303, 301 (67, 24%), 222 (25), 185 (26), 171(29), 100(74), 65(25), 63(56), 56(57), 36(100). Found $M^{+\cdot}$ 354.0124; 352.0123;

350.0189. $C_{11}H_{12}Cl_2N_4O_5$ requires 354.0126; 352.0155; 350.0185. Anal. ($C_{11}H_{12}Cl_2N_4O_5$) C,H,N,Cl.

- N,N-dimethyl [N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (9) (Ib: X = Cl, R = $CONMe_2$) (65% yield), mp ($CHCl_3$/petroleum ether) 130.5 °C. ^{1}H NMR ($CDCl_3$) δ 8.61 (s, 1 H, H-3), 7.07 (s, 1 H, H-6), 3.62 (s, 8 H, NCH_2CH_2Cl), 3.07, 2.78 (2 x 3 H, $CON(CH_3)_2$)). ^{13}C NMR δ 166.12 ($CON(CH_3)_2$)), 148.33 (C-5), 138.88, 135.75 (C-2,4), 137.82 (C-1), 124.92, 120.00 (C-3,6), 53.55 (CH_2N), 40.98 (CH_2Cl), 38.06, 34.85 ($CON(CH_3)_2$)). Anal. ($C_{13}H_{16}Cl_2N_4O_5$) C,H,N.

- N-morpholino [N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (10) (Ib: X = Cl, R = $CON(CH_2CH_2)_2O$), mp ($CHCl_3$/hexane) 140-142 °C. ^{1}H NMR ($CDCl_3$) δ 8.66 (s, 1 H, ArH-3), 7.55 (s, 1 H, ArH-6), 3.68 (br, 10H, $NC\underline{H}_2C\underline{H}_2Cl$ and $C\underline{H}_2O$), 3.60 & 3.34 (2 x m, 2 x 2 H, CH_2NCO). ^{13}C NMR δ 165.22 (CON), 149.34 (C-5), 139.88, 137.00 (C-2,4), 138.26 (C-1), 125.59, 121.17 (C-3,6), 66.92, 66.79 (CH_2O), 54.22 (CH_2N, 47.88, 42.80 ($CONH\underline{C}H_2$), 42.26 (CH_2Cl). Anal. ($C_{15}H_{18}Cl_2N_4O_6$) C,H,N.

- N-[2-(dimethylamino)ethyl] [N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (11) (Ib: X = Cl, R = $CONHCH_2CH_2NMe_2$), mp (hydrochloride salt from MeOH/EtOAc) 85-90 °C. ^{1}H NMR (D_2O) δ 8.76 (s, 1H, ArH-3), 7.59 (s, 1H, ArH-6), 3.86-3.80 (m, 10H, $NC\underline{H}_2C\underline{H}_2Cl$ and $C\underline{H}_2NH^+Me_2$), 3.53 t, J = 6.1 Hz, 2H, $CONHC\underline{H}_2$), 3.06 (s, 6H, NMe_2). ^{13}C NMR δ 170.77 ($\underline{C}$ONH), 166.12, 1451.39 (C-5), 140.53, 137.51 (C-2,4), 138.18 (C-1), 128.25, 123.39 (C-3,6), 58.46 ($\underline{C}H_2N^+HMe_2$), 55.49 (CH_2N), 45.67 (N^+HMe_2), 43.97 (CH_2Cl), 38.54 ($CONH\underline{C}H_2$). Anal. ($C_{15}H_{21}Cl_2N_5O_5$.HCl) C,H,N.

A solution of the free base of (11) (0.50 g, 1.18 mmol) in CH_2Cl_2 (15 mL) was treated with a solution of 2-benzenesulfonyl-3--phenyloxaziridine [Davis, F.; Stringer, C.D. *J. Org. Chem.*, 1982, *47*, 1775-1777]. (0.32 g, 1.24 mmol) in CH_2Cl_2 (3 mL) was added dropwise to. After 10min, petroleum ether (10 mL) was added, the solution was cooled at - 30 °C overnight, and the resulting solid was dried under

high vacuum to give N-(N^1,N^1-dimethylaminoethyl) [N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide N^1-oxide (**14**) ((Ib: X = Cl, R = $CONHCH_2CH_2N(O)Me_2$) as a hygroscopic foam. Treatment with MeOH/HCl gave the hydrochloride salt (0.36g, 64%), mp (MeOH/^{i}Pr$_2$O) 165-169°C. ^{1}H NMR ($D_2O/(CD_3)_2SO$) δ 8.82 (s, 1 H, H-3), 7.46 (s, 1 H, H-6), 3.98, 3.93 (2xt, J = 5.1 Hz, 2x2 H, CH_2CH_2), 3.82 (t, J = 4.7 Hz, 4 H, CH_2Cl), 3.77 (t, J = 4.7 Hz, 4 H, CH_2N), 3.57 (s, 6 H, $N(CH_3)_2$). ^{13}C NMR ($D_2O/(CD_3)_2SO$) δ 170.71 (CONH), 151.63 (C-5), 140.68, 137.31 (C-3,6), 69.20 (CH_2NOMe_2), 59.03 ($NOMe_2$), 55.40 (CH_2N), 44.06 (CH_2Cl), 36.45 (CONH CH_2). Anal. ($C_{15}H_{21}Cl_2N_5O_6$.HCl) C,H,N,Cl.

- N-[2-(morpholino)ethyl] [N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (12) (Ib: X = Cl, R = $CONHCH_2CH_2N(CH_2CH_2)_2O$) as a gum. The hydrochloride salt crystallized from MeOH/^{i}Pr$_2$O, mp 119-120 °C. ^{1}H NMR (D_2O) δ 8.81 (s, 1 H, H-3), 7.59 (s, 1 H, H-6), 4.07 (br, NH and HCl), 3.91 (t, J = 6.0 Hz, 4 H, $N^+CH_2CH_2O$), 3.86-3.81 (m, 10 H, NCH_2CH_2Cl and $CH_2CH_2N^+$), 3.56 (t, J = 6.0 Hz, 6 H, $N^+CH_2CH_2O$ and $CONHCH_2$). ^{13}C NMR δ 171.17 (CONH), 151.69 (C-5), 140.61, 137.28 (C-2,4), 138.12 (C-1), 128.53, 123.48 (C-3,6), 66.30 ($N^+CH_2C_H2O$), 58.07 (CH_2N^+), 55.48 (CH_2N), 54.65 ($N^+\underline{C}H_2CH_2O$), 44.09 (CH_2Cl), 36.84 ($CONH\underline{C}H_2$). Anal. ($C_{17}H_{23}Cl_2N_5O_6$.HCl.½H_2O) C,H,N,Cl.

- N-(2-hydroxyethyl) 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (**16**) (Ib: X = Cl, R = $CONHCH_2CH_2OH$) (1.24 g, 85%), mp (EtOAc/petroleum ether) 157-158 °C. ^{1}H NMR ($(CD_3)_2SO$) δ 8.73 t, J = 5.5 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.45 (s, 1 H, H-6), 4.76 (t, J = 5.5 Hz, 1 H, OH), 3.82 (t, J = 6.0 Hz, 4 H, CH_2Cl), 3.68 (t, J = 6.0 Hz, 4 H, CH_2N), 3.54 (dt, J = 6.1, 5.5 Hz, 2 H, CH_2OH), 3.31 (dt, J = 6.1, 5.5 Hz, 2 H, $CONHCH_2$). ^{13}C NMR ($(CD_3)SO$) δ 164.44 (CONH), 147.11 (C-5), 137.87. 136.27 (C-2,4), 137.32 (C-1), 124.24, 121.01 (C-3,6), 59.32 (CH_2OH), 52.44 (CH_2N), 41.93 ($CONHCH_2$), 41.65 (CH_2Cl). Anal. ($C_{13}H_{16}Cl_2N_2O_6$) C,H,N,Cl.

-N-(2,3-dihydroxypropyl)5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide(**17**) (Ib: X = Cl, R = $CONHCH_2CH(OH)CH_2OH$) (0.96 g, 59%) as a hygroscopic

yellow foam. ^1H NMR $((CD_3)_2SO)$ δ 8.70 (t, J = 5.7 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.44 (s, 1 H, H-6), 4.83 (d, J = 4.9 Hz, 1 H, CHO*H*) 4.56 (t, J = 5.7 Hz, 1 H, CH_2OH), 3.83 (t, J = 5.9 Hz, 4 H, CH_2Cl), 3.68 (t, J = 5.9 Hz, 4 H, CH_2N), 3.50-3.06 (m, 5 H, CONH $CH_2CHOHCH_2OH$). ^{13}C NMR $((CD_3)_2SO)$ δ 164.61 (CONH), 147.12 (C-5), 37.94, 136.40 (C-2,4), 137.40 (C-1), 124.29, 121.18 (C-3,6). 70.08 (CHOH), 63.77 (CH_2OH), 52.55 (CH_2N), 42.79 (CONHCH_2), 41.72. (CH_2Cl). Anal. ($C_{14}H_{18}Cl_2N_4O_7$) C,H,N,Cl.

- crude N-(2-methoxycarbonylethyl) 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide [from treatment with ß-alanine methyl ester hydrochloride] (Ib: X = Cl, R = $CONHCH_2CH_2COOMe$) as a yellow oil. This was immediately dissolved in THF (25 mL) and treated with aqueous KOH (10 mL of 2N) at 20 °C for 3h. The solution was then acidified with conc. HCl, extracted with EtOAc, and worked up as usual. Chromatography on silica, eluting with EtOAc, gave N-(2-carboxyethyl) 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (15) (Ib: X = Cl, R = $CONHCH_2CH_2COOH$) (0.72 g, 68%), mp (EtOAc/petroleum ether) 150-153°C. ^1H NMR $((CD_3)_2SO)$ δ 12.27 (br, 1 H, COOH) 8.79 (t, J = 5.6 Hz, 1 H, CONH), 8.54 (s, 1 H, H-3), 7.42 (s, 1 H, H-6) 3.83 (t, J = 6.0 Hz, 2 H, CH_2Cl), 3.68 (t, J = 6.0 Hz, 4 H, CH_2N), 3.44 (dxt, J = 6.9, 2 H, 5.6 Hz, CONHCH_2), 2.53 (t, J = 6.9 Hz, 2 H, CH_2, COOH). ^{13}C NMR $((CD_3)_2SO)$ δ 172.74 (COOH), 164.53 (CONH), 147.21 (C-5), 137.94, 136.13 (C-2,4), 137.29 (C-1), 124.37, 120.93 (C-3,6), 52.58 (CH_2N), 41.72 (CH_2Cl), 35.24, 33.19 (CONHCH_2CH_2). Anal. ($C_{14}H_{16}Cl_2N_4O_7$) C,H,N,Cl.

Example C: Preparation of 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzonitrile (7) (Ic: X = Cl, R = CN) and 5-[N,N-bis(2-chloroethyl)amino]-2,4--dinitrothiobenzamide (13) (Ic: X = Cl, R = $CSNH_2$) by the method outlined in Scheme 3.

A solution of 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (1) (VII) (0.20 g, 0.57 mmol) in $SOCl_2$ (5 mL) was heated under reflux in N_2 for 84 h. Excess $SOCl_2$ was removed under reduced pressure, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (3:7) gave 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzonitrile (7) (Ic: X = Cl, R = CN) (0.16 g, 87%), mp ($CHCl_3$) 127 °C. ^{1}H NMR (CD_3COCD_3) δ 8.79 (s, 1 H, H-3), 8.19 (s, 1 H, H-6), 3.93 (s, 8 H, NCH_2CH_2Cl). ^{13}C NMR δ 148.55 (C-5), 140.98, 139.07 (C-2,4), 129.50, 126.10 (C-3,6), 115.05 (C-1), 112.84 (CN), 54.18 (CH_2N), 42.20 (CH_2Cl). Anal. ($C_{11}H_{10}Cl_2N_4O_4$) C,H,N.

A solution of (1) (VII) (0.50 g, 1.42 mmol) in p-dioxane (20 mL) was treated with P_2S_5 (0.63 g, 2.84 mmol) and $NaHCO_3$ (0.24 g, 2.84 mmol) was heated under reflux with stirring for 3 h. The residue after workup was chromatographed on silica gel, and elution with EtOAc/petroleum ether (1:4) gave 5-[N,N-bis(2--chloroethyl)amino]-2,4-dinitrothiobenzamide (13) Ic: X = Cl, R = $CSNH_2$) (0.48 g, 92%), mp (EtOAc/petroleum ether) 166-167 °C. ^{1}H NMR (CD_3SOCD_3) δ 9.50, 9.34 (2xbr, 2 H, $CSNH_2$), 8.50 (s, 1 H, H-3), 7.45 (s, 1 H, H-6), 3.87, 3.82 (2xm, 8 H, NCH_2CH_2Cl). ^{13}C NMR δ 199.46 (CS), 148.08 (C-5), 143.87 (C-1), 139.51. 136.99 (C-2,4), 125.23, 121.36 (C-3,6), 54.23 (CH_2N), 42.02 (CH_2Cl). Anal. ($C_{11}H_{12}Cl_2N_4O_4S$) C,H,N,S.

Example D : Preparation of 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzenesulfonamide (18) (Id : X = Cl, R = SO_2NH_2) by the method outlined in Scheme 4.

A solution of 5-chloro-2,4-dinitrobenzenesulfonamide (VIII) [Herbert, R.B. and Hollman, R.G. *Tetrahedron*, 1965, *21*, 663-675] (1.55 g, 5.5 mmol) and diethanolamine (1.16 g, 11 mmol) in dioxan (100 mL) was held at 60 °C for 1.5 h, then adsorbed directly onto silica gel. Excess solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel, eluting with EtOAc/MeOH (19:1), to give 5-[N,N-bis(2-hydroxyethyl)amino]-2,4-dinitrobenzenesulfonamide (IX) (1.91 g, 99%), mp (EtOAc/petroleum ether) 138-139 °C. ^{1}H NMR $((CD_3)_2SO)$ δ 8.53 (s, 1 H, H-3), 7.97 (s, 1 H, H-6), 7,93 (br s, 2 H, SO_2NH_2), 4.88 (t, J = 5.1 Hz, 2 H, OH), 3.65 (dt, J = 5.2, 5.1 Hz, 4 H, CH_2OH), 3.49 (t, J = 5.1 Hz, 4 H, NCH_2). ^{13}C NMR δ 146.84 (C-5), 140.53 (C-1), 136.84, 134.39 (C-2,4), 125.70 (C-3), 54.27 (NCH_2). Anal. ($C_{10}H_{14}N_4O_8S$) C,H,N.

A stirred solution of the above diol (IX) (1.90 g, 5.43 mmol) and Et_3N (1.90 mL. 14 mmol) in dry THF (60 mL) was treated dropwise at 0 °C with methanesulfonyl chloride (0.89 mL, 11 mmol). After a further 15 min, the solution was diluted with EtOAc, washed well with water, and worked up to give the crude dimesylate (Id: X = OSO_2Me, R = SO_2NH_2). This was immediately dissolved in DMF (50 mL) containing LiCl (20 g), and the mixture stirred at 130 °C for 15 min before solvent was removed under reduced pressure. The residue was partitioned between EtOAc and water, and the organic layer was worked up and chromatographed on silica gel. Elution with EtOAc gave 5-[N,N-bis(2-chloroethyl)amino]-2,4-dinitrobenzenesulfonamide (18) (Id : X = Cl, R = SO_2NH_2) (1.47 g, 64%), mp 153-154 °C. (EtOAc/petroleum ether). ^{1}H NMR $((CD_3)_2CO)$ δ 8.54. (S, 1H, H-3), 7.99 (br, 2H, SO_2NH_2), 7.91 (S, 1H, H-6), 3.84 (t, J = 5.6 Hz, 4H, CH_2Cl), 3.67 (t, J = 5.6 Hz, 4H, CH_2N). ^{13}C NMR $((CD_3)_2CO)$ δ 146.13 (C-5), 140.64 (C-1), 138.86, 136.93 (C-2,4), 124.95, 121.77 (C-3,6), 52.47 (CH_2N), 41.62 (CH_2Cl). Anal. ($C_{10}H_{12}Cl_2N_4O_6S$) C,H,N,S.

Example E : Preparation of 2-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzamide (19) (Ie: R = $CONH_2$) and N-(N,N-dimethylaminoethyl) 2-[N,N-bis(2--chloroethyl)amino]-3,5-dinitrobenzamide (20) (Ie: R = $CONHCH_2CH_2NMe_2$) by the method outlined in Scheme 5.

Reaction of commercially-available 2-chloro-3,5-dinitrobenzoic acid (X) with $SOCl_2$ gave 2-chloro-3,5-dinitrobenzoyl chloride (XI: Z = Cl), which was quenched with ammonia to give 2-chloro-3,5-dinitrobenzamide (XI: Z = NH_2). A solution of this amide (1.00 g, 4.07 mmol) and Et_3N (1.42 g, 10 mmol) in *p*-dioxane (30 mL) was treated with N,N-bis(2-chloroethyl)amine hydrochloride (1.45 g, 8.14 mmol) at 50 °C for 18 h. The mixture was then poured into water and extracted with EtOAc to give an oil, which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:1) gave 2-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzamide (**19**) (Ie: R = $CONH_2$) (1.15 g, 80%), mp ($CHCl_3$/petroleum ether) 123-124 °C. ^{1}H NMR (CD_3SOCD_3) δ 8.70 (d, J = 2.7 Hz, 1 H, H-4), 8.49 (d, J = 2.7 Hz, 1 H, H-6), 7.68, 7.35 (2xbr, 2H, $CONH_2$), 3.80, 3.61 (2xt, J = 6.9 Hz, 8 H, NCH_2CH_2Cl). ^{13}C NMR δ 167.94 ($CONH_2$), 147.27 (C-2), 147.20, 142.84 (C-3,5), 137.70 (C-1), 128.40, 123.24 (C-4,6), 55.84 (CH_2N), 42.28 (CH_2Cl). Anal. ($C_{11}H_{12}Cl_2N_4O_5$) C,H,N,Cl.

Similar treatment of the acid chloride (XI: Z = Cl) with N,N--dimethylethylenediamine in water at 0 °C gave N-(N,N-dimethylaminoethyl 2-chloro-3,5-dinitrobenzamide (XI: Z = $NHCH_2CH_2NMe_2$) as a viscous oil (5.6 g, 59%. The hydrochloride salt crystallised from MeOH/isopropyl ether, mp 220-222 °C. ^{1}H NMR (D_2O) δ 8.99 (d, J = 2.3 Hz, 1 H, ArH4), 8.74 (d, J = 2.3 Hz. 1 H, ArH6), 3.89 (t, J = 6.3 Hz, 2 H, $CH_2N^+Me_2$), 3.51 (t, J = 6.3 Hz, 2 H, $CONHC\underline{H}_2$), 3.04 (s, 6 H, NMe_2). ^{13}C NMR 169.32 (CONH), 150.88 (C-2), 148.70, 141.02 (C-3,5), 133.23 (C-1), 129.45, 125.27 (C-4,6), 58.64 ($\underline{C}H_2N^+Me_2$), 45.88 (N^+Me_2), 37.94 ($CONH\underline{C}H_2$). Anal. ($C_{11}H_{13}ClN_4O_5$.HCl) C,H,N,Cl.

Reaction of this amide (XI: Z = $NHCH_2CH_2NMe_2$) with bis(2-chloroethyl)amine hydrochloride and Et_3N as above, followed by chromatography on silica gel and elution with EtOAc/MeOH (9:1) gave N-(N,N-dimethylaminoethyl) 2-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzamide (20) (Ie: R = $CONHCH_2CH_2NMe_2$), mp (EtOAc/petroleum ether) 115-118 °C. ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 2 H, ArH4), 7.41 (br, 1 H, CONH), 3.74 (t, J = 6.3 Hz, 4 H, CH_2Cl), 3.59 (t, J = 5.8 Hz, 2 H, $CONHC\underline{H}_2$), 3.55 (t, J = 6.3 Hz, 4 H, $NC\underline{H}_2CH_2Cl$), 2.56 (t, J = 5.8 Hz, 2 H, CH_2NMe_2), 2.27 (s, 6 H, NMe_2). ^{13}C NMR 164.70 (CO), 145.93 (C-2), 144.86, 141.94 (C-3,5), 135.68 (C-1), 129.63 (C-4), 123.16 (C-6), 57.51 ($CONH\underline{C}H_2$), 54.82 ($N\underline{C}H_2CH_2Cl$), 45.12 (NMe_2), 41.39 ($NCH_2\underline{C}H_2Cl$), 37.67 ($CONH\underline{C}H_2$). Anal. ($C_{15}H_{21}Cl_2N_5O_5$) C,H,N,Cl. (The hydrochloride salt was too hygroscopic to handle).

Example F : Preparation of 3-[N,N-bis(2-chloroethyl)amino]-2,6-dinitrobenzamide (21) (If: R = $CONH_2$) and N-(N,N-dimethylaminoethyl) 3-[N,N-bis-(2-chloroethyl)amino]-2,6-dinitrobenzamide (22) (If: R = $CONHCH_2CH_2NMe_2$) by the method outlined in Scheme 6.

A solution of 3-chlorobenzoic acid (60 g, 0.38 mol) in c.H_2SO_4 (600 ml) was treated portionwise with fuming nitric acid (d 1.42) (150 ml), and the solution was warmed gradually with stirring to 140 °C in an open flask, and held at this temperature for 6h. After cooling overnight, ice-water was added cautiously, and after chilling to 5 °C for 3h the precipitated product was removed by filtration and washed well with water. Crystallisation from aqueous EtOH gave 5-chloro-2,4-dinitrobenzoic acid (II: Y = OH) (62 g, 66%) mp 180-183 °C [Goldstein, H.; Stamm, R. *Helv. Chim. Acta.*, 1952, *35*, 1330-1332 report mp 182-183 °C]. The original filtrate and washings were combined and allowed to stand for several hours, depositing crystals of pure 3--chloro-2,6-dinitrobenzoic acid (XII) (8.4 g, 9 %), mp (H_2O) 162-163.5 °C. ^{1}H NMR (CD_3SOCD_3) δ 10.50 (br, 1 H, COOH), 8.82 (d, J = 8.8 Hz, 1 H, H-5), 8.62 (d, J = 8.8 Hz, 1 H, H-4). ^{13}C NMR δ 162.03 (COOH), 146.34 (C-2), 145.57 (C-6), 133.69 (C-5), 130.01 (C-1), 128.26 (C-4), 125.19 (C-3). Anal. ($C_7H_3ClN_2O_6$) C,H,N,Cl.

The 3-chloro-2,6-dinitrobenzoic acid (XII) was converted to the ethyl ester, which was reacted as above with diethanolamine to give crude ethyl 3-[N,N-bis-(2-hydroxyethyl)amino]-2,6-dinitrobenzoate (XIII: X = OH, Z =OEt). Reaction of this with MsCl/LiCl gave ethyl 3-[N,N-bis(2-chloroethyl)amino]-2,6--dinitrobenzoate (XIII: X = Cl, Z = OEt), mp (EtOAc/petroleum ether) 84-87 °C. ^{1}H NMR (CD_3COCD_3) δ 8.32 (d, J = 9.4 Hz, 1 H, H-5), 7.79 (d, J = 9.4 Hz, 1 H, H-4) 4.39 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 3.85-3.77 (m, 8 H, NCH_2CH_2Cl), 1.34 (t, J = 7.1 Hz, 3 H, OCH_2CH_3). ^{13}C NMR δ 163.24 (COOEt), 148.30 (C-3), 141.04, 138.80 (C-2,6), 128.84, 124.65 (C-4,5), 127.39 (C-1), 63.94 (OCH_2CH_3), 54.39 (NCH_2), 42.00 (CH_2Cl) 13.89 (OCH_2CH_3). Anal. ($C_{13}H_{15}Cl_2N_3O_6$) C,H,N,Cl.

An X-ray crystallographic determination was carried out on this compound to confirm the structure.

Hydrolysis of this ester (XIII: X = Cl, Z = OEt) in aqueous KOH/*p*-dioxane (30 mL) at 20 °C for 18 h gave 3-[N,N-bis(2-chloroethyl)amino]-2,6--dinitrobenzoic acid (XIII: X = Cl, Z = OH), which was converted to the corresponding acid chloride (XIII: X = Cl, Z = Cl). A solution of this in Me_2CO at 0 °C was treated with excess aqueous ammonia, and the product was chromatographed on silica gel, EtOAc/petroleum ether (1:1) eluting 3-[N,N-bis(2--chloroethyl)amino]-2,6-dinitrobenzamide (21) (If: R = $CONH_2$) (0.93g), mp (EtOAc/petroleum ether) 140-141.5°C. ^{1}H NMR (CD_3COCD_3) δ 8.26 (d, J = 9.3 Hz, 1 H, H-5), 7.71 (d, J = 9.3 Hz, 1 H, H-4), 7.63, 7.23 (2xbr, 2 H, $CONH_2$), 3.75 (s, 8 H, NCH_2CH_2Cl). ^{13}C NMR δ 163.99 ($CONH_2$), 147.38 (C-3), 142.92, 139.76 (C-2,6), 130.48 (C-1), 128.22, 124.23 (C-4,5), 54.74 (NCH_2), 42.00 (CH_2Cl). Anal. ($C_{11}H_{12}Cl_2N_4O_5$) C,H,N,Cl.

Similar treatment of the acid chloride (XIII: X = Cl, Z = Cl) with N,N--dimethylethylenediamine gave N-(N,N-dimethylaminoethyl) 3-[N,N-bis(2--chloroethyl)amino]-2,6-dinitrobenzamide (22) (If: R = $CONHCH_2CH_2NMe_2$). The hydrochloride salt crystallised from MeOH/isopropyl ether, mp 180-182 °C. 1H NMR (D_2O) δ 8.37 (d, J = 9.6 Hz, 1 H, ArH5), 7.63 (d, J = 9.6 Hz, 1 H, ArH4), 3.80-3.71 (m, 10 H, $NC\underline{H}_2C\underline{H}_2Cl$ and $C\underline{H}_2NMe_2$), 3.44 (t, J = 6.4 Hz, 2 H,

$CONHC\underline{H}_2$), 3.00 (s, 6 H, NMe_2). ^{13}C NMR δ 168.75 (CONH), 150.91 (C-3), 141.55, 138,72 (C-2,6), 131.61 (C-4), 130.45 (C-1), 125.85 (C-5), 58.31 ($\underline{C}H_2N^+Me_2$), 55.74 ($NCH_2\underline{C}H_2Cl$), 45.97 (N^+Me_2), 44.16 ($NCH_2\underline{C}H_2Cl$), 37.91 ($CONH\underline{C}H_2$). Anal. ($C_{15}H_{21}Cl_2N_5O_5$.HCl) C,H,N,Cl.

Example G : Preparation of 4-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzamide (23) (Ig: X = Cl, R = $CONH_2$) and N-(N,N-dimethylaminoethyl) 4-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzamide (24) (Ig: X = Cl, R = $CONHCH_2CH_2NMe_2$) by the method outlined in Scheme 7.

A solution of methyl 4-chloro-3,5-dinitrobenzoate (XIV) (10.0 g, 0.036 mol) and diethanolamine (7.66 g, 0.073 mol) in *p*-dioxane (60 mL) was stirred at 50 °C for 5h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (1:10) gave foreruns, and subsequent elution with EtOAc/petroleum ether (3:2) gave methyl 4-[N,N-bis(2-hydroxyethyl)amino]-3,5-dinitrobenzoate (XV: X = OH, Z = OMe) (9.61 g, 80%), mp ($CHCl_3$/petroleum ether) 101-103° C. ^1H NMR ($CDCl_3$) δ 8.52 (s, 2 H, ArH-2,6), 3.97 (s, 3 H, $COOCH_3$), 3.76 (dxt, *J* = 6.5, 4.7 Hz, 4 H, CH_2OH), 3.26 (t, *J* = 4.7 Hz, 4 H, NCH_2), 2.76 (t, *J* = 6.5 Hz, 2 H, OH). ^{13}C NMR δ 163.14 ($COOCH_3$), 145.50 (C-4), 142.54 (C-3,5), 131.19 (C-2,6), 123.59 (C-1), 59.16 (CH_2OH)1, 54.58 (CH_2N), 53.14 (OCH_3). Anal. ($C_{12}H_{15}N_3O_8$) C,H,N.

MsCl (3.93 mL, 0.049 mol) was added dropwise at 0 °C to a solution of the above diol (XV: X = OH, Z = OMe) (7.30 g, 0.022 mol) and Et_3N (7.72 mL, 0.055 mol) in CH_2Cl_2 (100 mL). After 15 min, the solution was washed several times with water and worked up to give the crude dimesylate, which was treated with LiCl in DMF at 120 °C for 5 min. Workup and chromatography on silica gel (elution with EtOAc/petroleum ether (1:4)) gave methyl 4-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzoate (XV: X = Cl, Z = OMe) (6.21 g, 77%), mp ($CHCl_3$/petroleum ether) 93-95 °C. ^{1}NMR ($CDCl_3$) δ 8.54 (s, 2 H, H-2,6), 3.99 (s, 3 H, $COOCH_3$), 3.63 (t, *J* = 6.9 Hz, 4 H, CH_2Cl), 3.43 (t, *J* = 6.9 Hz, 4 H, CH_2N). ^{13}C NMR δ

162.90 ($COOCH_3$), 147.83 (C-4), 140.53 (C-3,5), 129.85 (C-2,6), 126.70 (C-1), 55.47 (CH_2N), 53.23 (OCH_3), 41.18 (CH_2Cl). Anal. ($C_{12}H_{13}Cl_2N_3O_6$) C,H,N,Cl.

Hydrolysis of this ester (XV: X = Cl, Z = OMe) with aqueous KOH/*p*-dioxane at 20 °C for 3 h gave a quantitative yield of 4-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzoic acid (XV: X = Cl, Z = OH), mp EtOAc/petroleum ether) 164-166 °C. ^{1}NMR (($(CD_3)_2SO$) δ 8.61 (s, 2 H, H-2,6), 3.78 (t, J = 6.9 Hz, 4 H, CH_2Cl), 3.52 (t, J = 6.9 Hz, 4 H, CH_2N) (COOH not visible). ^{13}C NMR δ 164.12 (COOH), 149.12 (C-4), 141.40 (C-3,5), 130.70 (C-2,6), 128.19 (C-1), 56.14 (CH_2N), 42.46 (CH_2Cl). Anal ($C_{11}H_{11}Cl_2N_3O_6$) C,H,N,Cl.

The above acid (XV: X = Cl, Z = OH) was converted to the crude acid chloride (XV: X = Cl, Z = Cl), which was dissolved in dry Et_2O (100 mL), cooled to 5 °C, and treated dropwise with aqueous NH_3 (20 mL of a 3N solution, 60 mmol). After 10 min the solution was worked up, and the residue was chromatographed on silica. Elution with EtOAc/petroleum ether (3:7) gave foreruns, while EtOAc/petroleum ether (7:3) gave 4-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzamide (**23**) (Ig: X = Cl, R = $CONH_2$) (80% overall yield), mp (EtOAc/petroleum ether) 113-116 °C. ^{1}H NMR (CD_3COCD_3) δ 8.61 (s, 2 H, H-2,6), 7.98, 7.24 (2xbr, 2 H, $CONH_2$), 3.75 (t, J = 7.0 Hz, 4 H, CH_2Cl), 3.50 (t, 4 H, CH_2N). ^{13}C NMR δ 164.91 ($CONH_2$), 149.32 (C-4), 140.07 (C-3,5), 132.18 (C-1), 128.91 (C-2,6), 56.37 (CH_2N), 42.55 (CH_2Cl). Anal. ($C_{11}H_{12}Cl_2N_4O_5$) C,H,N,Cl.

Similar treatment of the crude acid chloride (XV: X = Cl, Z = Cl) with N,N-dimethylethylenediamine gave N-(N,N-dimethylaminoethyl) 4-[N,N-bis(2-chloroethyl)amino]-3,5-dinitrobenzamide (**24**) (Ig: X = Cl, R = $CONHCH_2CH_2NMe_2$). The hydrochloride salt crystallised from MeOH/isopropyl ether, mp 145-148 °C. ^{1}H NMR (CD_3SOCD_3) δ 10.65 (br, 1 H, HCl), 9.49 (t, J = 5.4 Hz, 1 H, CONH), 8.75 (s, 2 H, ArH2,6), 3.71 (t, J = 6.9 Hz, 4 H, $NC\underline{H}_2CH_2Cl$), 3.44-3.28 (m, 8 H, $NCH_2C\underline{H}_2Cl$ and $CH_2CH_2N^+Me_2$), 2.83 (s, 6 H, N^+Me_2). ^{13}C NMR δ 162.63 (CO), 147.43 (C-3,5), 138.75 (C-4), 130.22 (C-1),

128.31 (C-2,6), 55.44 ($\underline{C}H_2N^+Me_2$), 54.75 ($N\underline{C}H_2CH_2Cl$), 42.22 (N^+Me_2), 42.18 ($NCH_2\underline{C}H_2Cl$), 34.75 ($CONH\underline{C}H_2$). Anal. ($C_{15}H_{21}Cl_2N_5O_5.HCl$) C,H,N,Cl.

The following Table 2 gives biological data for selected examples of the compounds listed in Table 1. The abbreviations used in Table 2 are :

No. The number given the corresponding compound in Table 1.

IC_{50} Growth inhibition studies were performed as described in detail elsewhere (W.R. Wilson, R.F. Anderson and W.A. Denny. *J. Med. Chem.*, **1989**, *32*, 23; G.J. Finlay, B.C. Baguley and W.R. Wilson. *Anal. Biochem.*, **1984**, *139*, 172.), using 200 viable AA8 or 300 viable UV4 cells plus 5000 lethally-irradiated AA8 feeder cells per well in 96-well tissue culture dishes. AA8 and UV4 cells were maintained in logarithmic-phase growth in 25 cm^3 tissue culture flasks with subculture twice weekly by trypsinization. The growth medium was antibiotic-free α-MEM with 10% v/v heat-inactivated (56°C, 40 min) fetal calf serum. Doubling times were approximately 14 h for AA8 and 15h for UV4 cells. Cultures were tested for mycoplasma contamination frequently, using a cytochemical staining method (I.R. Chen, *Exp. Cell Res.*, **1977**, *104*, 255. Drugs were added 24 hours after initiating cultures in 96-well dishes. and cells were incubated under aerobic or hypoxic conditions for 18 hours before washing with fresh medium. The IC_{50} was determined as the drug concentration needed to reduce the cell mass (protein content, measured after 72-78h by staining with methylene blue and measuring absorbance in a microplate photometer) to 50% of the mean value for 8 control cultures on the same 96-well plate.

HF_{air} Ratio of IC_{50} values for a compound after exposure as detailed above against AA8 and UV4 cell lines ($HF = IC_{50}(AA8)/IC_{50}(UV4)$).

CT_{10} The product (in μM-hr) of the drug concentration times the exposure time to reduce cell survival to 10% of control values in the clonogenic assay.

air/N_2 — Ratio of CT_{10} values after exposure of UV4 cells as detailed above under either aerobic (air) or hypoxic (N_2) conditions (= aerobic CT_{10}/hypoxic CT_{10}).

HF_{NR-2} — Ratio of IC_{50} values for a compound after exposure as detailed above against the UV4 cell line in the presence and absence of the E. coli nitroreductase enzyme NR-2.

Table 2: Biological activity of selected compounds of Table I

No	IC_{50} (AA8) (air : μM)	HF (air)	CT_{10} (UV4) (air : μM-h)	CT_{10} ratio (air/N_2)	IC_{50} (UV4) (air : μM)	HF (NR-2) (air)
1	468	6.8	2925	58	109	73.5
2	57	7.5	160	20	10.9	32.3
3	34	2.6	170	42	15	73.8
4	>200	-	1200	17	99	10.0
6	10600	1.5	>30000	>2	317	0.76
7	24	6.8	86	8.5	2.3	9.2
8	>100	-	>360	-		
9	310	1.4	>3500	>20		
10	264	23	156	1.6		
11	230	4.2	1130	60		
12	920	3.0	3700	45		
13	>25	>2	>60	>3		
14	7300	1.7	<14400	<10		
15	6100	1.1	14000	2		
17	1790	3.2	3340	14		
18	3	-	-	-		
19	>100	ca. 3	>300	>5		
20	150	14	1200	36		
21	>240	>2	>300	>3		
22	426	3.2	3600	130		
23	97	1.4	>450	>1		
24	62	2.0	>273	>1		

The following Examples 1 to 3 show that compound 1 (referred to therein as SN23862) is reductively activated by bacterial nitroreductase enzymes.

The alkylating agent 5-(aziridin-1-yl)-2,4-dinitrobenzamide (hereinafter designated CB 1954) has been known, almost for 20 years, as an interesting experimental compound of unique selectivity. Although CB 1954 is structurally quite closely related to numerous other known alkylating agents which have a relatively broad range of activity, CB 1954 exhibits considerable activity against the Walker tumour cells in vivo or in vitro but was thought to be virtually inactive against other tumours.

It was recently discovered that the selectivity of CB 1954 arose from the fact that it was not an anti-tumour agent per se but was a prodrug for an anti-tumour agent generated from CB 1954 by a nitroreductase enzyme found in the Walker cell. This nitroreductase from the Walker cell was subsequently shown to be an enzyme known from other sources which was an NAD(P)H dehydrogenase (quinone) classified as EC.1.6.99.2, see Robertson et al, *J. Biol. Chem.* 261, 15794-15799 (1986).

In the course of the previous investigations with CB 1954, it was found that the Walker cell enzyme EC.1.6.99.2 had the ability to reduce the 4-nitro group of CB 1954 to the corresponding hydroxylamine and that it was the resulting 5-(aziridin-1-yl)-2-nitro-4-hydroxylamino-benzamide that was the active anti-tumour agent.

PCT Application PCT/GB92/01947 of Cancer Research Campaign Technology Limited describes new nitroreductases, obtainable from bacterial sources, for example from *Escherichia coli* and from *Bacillus amyloliquifaciens*, that are of interest in that not only are they capable of converting CB 1954 into an active anti-tumour agent, but also, unlike EC.1.6.99.2. capable of converting CB 1954 analogues which are also prodrugs into active anti-tumour agents.

As is more particularly described in PCT Application PCT/GB92/01947 the use of prodrugs represents a clinically very valuable concept in cancer therapy since, particularly where the prodrug is to be converted to an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated antigen, the combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent.

Example 1.

The effect of SN23862 on the survival of V79 cells in the presence of the *E. coli* nitroreductase. All treatments were for 2 hours at 37°C and the cells were then plated out for their resulting colony forming ability. The nitroreductase concentration was 2μg/ml and NADH was used as a co-factor. The initial cell density was 2×10^5/mL.

TREATMENT	%SURVIVAL	% DRUG REDUCTION
CONTROL	100	-
+ 500μM NADH	100	-
+ 50μM SN23862	100	< 1.0
+ NADH + SN23862	100	< 1.0
+ Nitroreductase	94	-
+ NR + 50μM SN23862	99	< 1.0
+ NR + SN23862 + 500μM NADH	0.007	95

Example 2.

The effect of SN23862 on the survival of V79 cells in the presence of the *B. amyloliquifaciens* nitroreductase. All treatments were for 2 hours at 37°C and the cells were then plated out for their resulting colony forming ability. The nitroreductase concentration was 7.45μg/ml and NADH was used as a co-factor. The initial cell density was $2x10^5$/mL.

TREATMENT	%SURVIVAL	% DRUG REDUCTION
CONTROL	100	-
+ 500μM NADH	120	-
+ 50μM SN23862	110	<1.0
+ NADH + SN23862	115	<1.0
+ Nitroreductase (BA)	96.8	-
+ BA + 50μM SN23862	110	<1.0
+ BA + SN23862 + 500μM NADH	6.0	>95

Example 3.

The effect of SN23862 on the survival of V79 or Mawi cells in the presence of the *E. coli* nitroreductase: A5B7 $F(ab)_2$ conjugate. All treatments were for 2 hours at 37°C and the cells were then plated out for their resulting colony forming ability. The conjugate concentration was 81μg/ml (equivalent to 5.2μg/ml nitroreductase) and NADH was used as a co-factor. The initial cell density was $2x10^5$/mL. No attempt was made to remove unbound conjugate.

TREATMENT	%SURVIVAL		% DRUG REDUCTION
	MAWI Cells	V79 Cells	
CONTROL	100	100	-
+ 500μM NADH	104.6	94.6	-
+ 50μM SN23862	90.9	94.3	<1.0
+ NADH + SN23862	93.1	80.4	<1.0
+ Conjugate (CG)	85.5	85.8	-
+ CG + 50μM SN23862	94.6	73.5	<1.0
+ CG + SN23862 + 500μM NADH	0.014	0.003	>99

The accompanying Figure 1 shows radiosensitising properties of compound 1.

The radiosensitising efficiency of compound 1 has been assessed in stirred suspensions of AA8 cells under hypoxic conditions using gamma irradiation (cobalt 60) at a dose rate of 2.2 Gy/min at 37 °C. Drug and cells were mixed under hypoxic conditions 30 min before irradiation. Radiosensitisation of hypoxic cells was observed (Figure 1). The concentration for an enhancement ratio (ratio of radiation doses for 10% survival with and without drug) of 1.6 ($C_{1.6}$) was approximately 100μM, and this extent of sensitisation was observed in the absence of drug toxicity (Figure 1). Compound 1 is therefore a hypoxic cell radiosensitiser as well as a hypoxia-selective cytotoxin.

It is clear from the data of Table 2, Examples 1 to 3 and Figure 1 that the examples of the nitroaniline derivatives of general formula (I) listed in Table 2 include compounds which are active as cytotoxic agents, and which have the additional capability of being reductively activated by both mammalian tumour cells and bacterial nitroreductase enzymes, and are therefore suitable as prodrugs. The compounds are selectively toxic to hypoxic tumour cells, and therefore useful as anticancer drugs. The compounds are capable of sensitising hypoxic cells in vitro to ionising radiation, and thus are suitable as hypoxic cell radiosensitisers.

The present invention therefore also provides pharmaceutical compositions, having antitumour and radiosensitising activity, and comprising at least one compound represented by the general formula (I), and one or more pharmaceutically-acceptable carriers or diluents.

The present invention further provides the use of at least one compound represented by the general formula (I) for the manufacture of pharmaceutical compositions for the treatment of tumours, in particular cancers.

When the compounds of formula (I) are used as reductively-activated prodrugs for cytotoxins, they can be used in association with nitroreductase enzymes, including the nitroreductases described in Application PCT/GB92/01947, and so provide a system of cancer chemotherapy where the extent of exposure of the patient to the cytotoxic agent is limited, so far as possible, to those regions where there is the inter-reaction between the prodrug and the nitroreductase.

The most or one of the most convenient ways of utilising such a system of cancer chemotherapy is to conjugate the nitroreductase to a targetting agent such as a monoclonal antibody that will bind with a tumour-associated antigen.

The term "monoclonal antibody" will be understood by those of skill in the art not simply to refer to antibodies produced by traditional hybridoma techniques, but also to cover antibodies and variants thereof produced by recombinant means. These include, for example, humanised antibodies such as those with a constant region from a human antibody grafted onto a non-human antibody variable region (see for example EP-A-0 120 694), chimeric antibodies such as those with non-human complementarity determining regions (CDRs) grafted into a human variable region framework (see for example EP-A-0 239 400) and single chain antibodies. Fragments of such monoclonal antibodies which retain their target binding activity are also included by the general term "monoclonal antibody". This includes Fab' and $F(ab')_2$ fragments.

The selection of monoclonal antibody will clearly be influenced by the nature of the target tumour but for the purposes of illustration, reference may be made to the anti-CEA antibody A_5B_7.

With this system, it is possible in a course of cancer chemotherapy to administer to the patient requiring the treatment the nitroaniline compound which is the prodrug for a cytotoxic compound and the enzyme/targeting agent conjugate. The prodrug and the conjugate can be administered simultaneously but it is often found preferable, in clinical practice, to administer the enzyme/agent conjugate before the

prodrug; e.g. up to 72 hours before, in order to give the enzyme/agent conjugate an opportunity to localise in the region of the tumour target. By operating in this way, when the prodrug is administered, conversion of the prodrug to the cytotoxic agent tends to be confined to the regions where the enzyme/agent conjugate is localised, i.e. the region of the target tumour and damage to healthy cells caused by the premature release of the cytotoxic agent is minimised.

The degree of localisation of the enzyme/agent conjugate (in terms of the ratio of localized to freely circulating active conjugate) can be further enhanced using the clearance and/or inactivation systems described in WO89/10140. This involves, usually following administration of the conjugate and before administration of the prodrug, the administration of a component (a "second component") which is able to bind to the such part of the conjugate so as to inactivate the enzyme and/or accelerate the clearance of the conjugate from the blood. Such a component may include an antibody to the nitroreductase which is capable of inactivating the enzyme.

The second component may be linked to a macromolecule such as dextran, a liposome, albumin, macroglobulin or a blood group O erythrocyte so that the second component is restrained from leaving the vascular compartment. In addition or as an alternative, the second component may include a sufficient number of covalently bound galactose residues, or residues of other sugars such as lactose or mannose, so that it can bind the conjugate in plasma but be removed together with the conjugate from plasma by receptors for galactose or other sugars in the liver. The second component should be administered and designed for use such that it will not, to any appreciable extent, enter the extravascular space of the tumour where it could inactivate localised conjugate prior to and during administration of the prodrug.

The exact dosage regime will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact nature of the prodrug and the cytotoxic agent to be released from the prodrug but

some general guidance can be given. Chemotherapy of this type will normally involve parenteral administration of both the prodrug and the enzyme/agent conjugate and administration by the intravenous route is frequently found to be the most practical.

The active compounds may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 and about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 5 and about 200 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course any

material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparations and formulations.

The active compounds may also be administered parenterally or intraperitoneally. Solutions of the active compound as a free base or pharmaceutically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against be contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, clorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

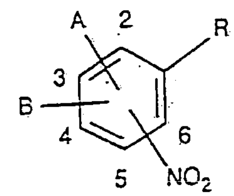
It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suitabale as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically-acceptable

carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from about 0.1 to about 400 mg, with from about 1 to about 30 mg being preferred. Expressed in proportions, the active compound is generally present in from about 0.1 to 400 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

WHAT WE CLAIM IS :

1. A compound represented by the general formula (I),



(I)

where the nitro group is substituted at any one of the available benzene positions 2-6; where R and A separately represent the groups NO_2, CN, $COOR^1$, $CONR^1R^2$, $CSNR^1R^2$ or $SO_2NR^1R^2$ and A is substituted at any one of the available benzene positions 2-6; where B represents $N(CH_2CH_2halogen)_2$ or $N(CH_2CH_2OSO_2R^3)_2$ substituted at any one of the available benzene positions; and where R^1, R^2 and R^3 separately represent H, or lower alkyl optionally substituted with hydroxyl, ether, carboxy or amino functions, including cyclic structures, or R^1 and R^2 together with the nitrogen form a heterocyclic structure.

2. A compound of formula (I) according to claim 1, where NO_2 is in the 2-position, R represents $CONH_2$, A represents 4-NO_2 and B represents 5-$N(CH_2CH_2Cl)_2$.

3. A compound of formula (I) according to claim 1, where NO_2 is in the 2-position, R represents $CONH_2$, A represents 4-NO_2 and B represents 5-$N(CH_2CH_2Br)_2$.

4. A compound of formula (I) according to claim 1, where NO_2 is in the 2-position, R represents $CONH_2$, A represents 4-NO_2 and B represents 5-$N(CH_2CH_2I)_2$.

5. A compound of formula (I) according to claim 1, where NO_2 is in the 2-position, R represents $CONHCH_2CH_2NMe_2$, A represents 4-NO_2 and B represents 5-$N(CH_2CH_2Cl)_2$.

6. A compound of formula (I) according to claim 1, where NO_2 is in the 2-position, R represents $CONHCH_2CH_2N(O)Me_2$, A represents 4-NO_2 and B represents 5-$N(CH_2CH_2Cl)_2$.

7. A compound of formula (I) according to claim 1, where NO_2 is in the 2-position, R represents $CONH_2$, A represents 6-NO_2 and B represents 5-$N(CH_2CH_2Cl)_2$.

8. A compound of formula (I) according to claim 1, where NO_2 is in the 2-position,R represents $CONHCH_2CH_2NMe_2$, A represents 6-NO_2 and B represents 5-$N(CH_2CH_2Cl)_2$.

9. A compound of formula (I) according to claim 1, where NO_2 is in the 3-position,R represents $CONH_2$, A represents 5-NO_2 and B represents 2-$N(CH_2CH_2Cl)_2$.

10. A compound of formula (I) according to claim 1, where NO_2 is in the 3-position,R represents $CONHCH_2CH_2NMe_2$, A represents 5-NO_2 and B represents 2-$N(CH_2CH_2Cl)_2$.

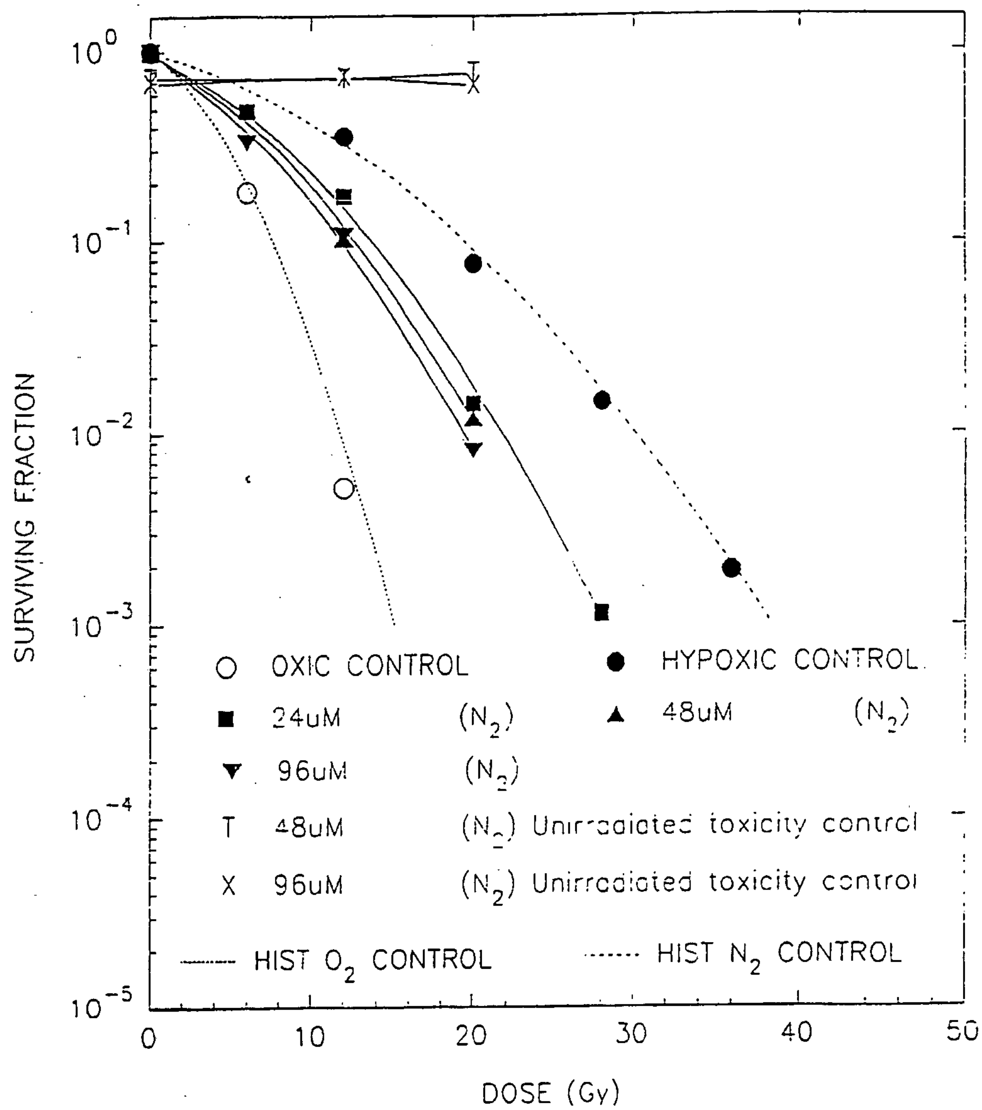
11. A process for the preparation of a compound represented by the general formula (I) as defined in claim 1, which process is outlined at any one of Schemes 1-7 herein.

12. A compound represented by the general formula (I) as defined in claim 1, whenever prepared by a process outlined in claim 11, or by an obvious chemical equivalent thereof.

13. A pharmaceutical composition with comprises at least one compound represented by the general formula (I) as defined in claim 1, and one or more pharmaceutically-acceptable carriers or diluents.

14. The use of at least one compound represented by the general formula (I) as defined in claim 1 for the manufacture of pharmaceutical compositions for the treatment of humans.

FIGURE 1. Radiosensitising properties of compound 1



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/02199

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07C237/30; A61K31/165; C07C237/32; C07C237/34
C07C237/36; C07C229/60; A61K31/195; C07C255/50

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07C ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 330 432 (THE DEPARTMENT OF MOLECULAR PHARMACOLOGY, INSTITUTE OF CANCER RESEARCH) 30 August 1989 see the whole document ---	1,13
A	CHEMICAL ABSTRACTS, vol. 66, no. 1, 2 January 1967, Columbus, Ohio, US; abstract no. 10687m, Y. M. ABOU-ZEID ET AL. 'Synthesis of 2,4-dinitrophenol derivatives as antineoplastic agents' page 1021 ; see abstract & J. PHARM. SCI U. ARAB REP. vol. 5, 1964, pages 133 - 138 ---	1,13

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° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

1

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20 JANUARY 1993	18.02.93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SEUFERT G.H.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category°	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 75, no. 7, 16 August 1971, Columbus, Ohio, US; abstract no. 48780e, J. M. TISDALE 'Alkylating Analogs of the Tumour Inhibitor 5-Aziridino-2,4-dinitrobenzamide' page 339 ; see abstract & CHEM. BIOL. INTERACTIONS vol. 3, no. 2, 1971, pages 95 - 107	1,13
A	EP,A,0 270 292 (MERCK & CO INC.) 8 June 1988 see page 2, line 6 - line 9; claims	1,13
P,X	JOURNAL OF MEDICINAL CHEMISTRY vol. 35, no. 17, WASHINGTON US pages 3214 - 3222 B. D. PALMER ET AL. 'Hypoxia-Selective Antitumour Agents. 5. Synthesis of Water-Soluble Nitroaniline Mustards with Selective Cytotoxicity for Hypoxic Mammalien Cells' see table II, no. 20 - 22; page 3218, right column, last paragraph - page 3219, right column, third paragraph	1,2,5,13

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9202199
SA 67004

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 20/01/93
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP-A- 0429454	05-06-91
		WO-A- 8907592	24-08-89
		JP-T- 3503761	22-08-91
EP-A-0270292	08-06-88	JP-A- 63154654	27-06-88
		US-A- 4853415	01-08-89

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82